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Antioxidant activity of Lawsoniainermis Stem

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ABSTRACT

The present investigation shows the Micromarphlogical characters of Lawsonia inermis stem.The preliminary phytochemical screening of this studies shows the main chemical constituents of Lawsonia inermis stem are Alkaloids, Carbohydrates, glycosides, fixed oil & fats, flavonoids.This Lawsonia inermis stem is charecterized by the presence of trichomes. Epidermis is the outermost layer and consists of single layer epidermis of quadrangular cells.The outer walls of the cells are cutinised. From some of the cells multicellular, uniseriate hairs called epidermal hairs,(trichomes) are found. Stomata are present. Cortex is well developed with collenchymotous , general cortex , hypodermis & endodermis as starch sheath . Pericycle is represented by semilunar patches of sclerenchyma with intervening masses of parenchyma.Vascular bundles are limited in number arranged in a ring & are wedge or top shaoed and open.Xylem vessels are more in number & arranged in serial order. Medulla and medullary rays are present. From maceration method ethanol extract is more. Soxhlation extraction method shows the plant material that could has no known anti-microbial effect (for example a carrier oil such as sunflower oil) at the testing stages. Phytochemical investigation of stem is done with the ethanol extract. In ethanol extract good quantity of alkaloids, acid ,coumarin ,carbohydrate, glycosides, fixed oils&fats, flavonoids, furonoids, phenols, phytosterols, triterpenoids, tannins, sterols saponins are present.High performenced Liquid chromatography as been analyzed. UV spectroscopy showed best results on stem extrat. Invitro Antioxidant studies as been done. In vivo Anti-inflammatory activity shows the application of LI stem extract resulted in significantly ($p<0.01$) more % inhibition of swelling of rat paw edema (37.68±0.73%) when compared to marketed gel (28.98±0.68%) at the end of 4h.

***Keywords*:** stem, lawsonia inermis, extract, Antioxidant etc…

INTRODUCTION

Antioxidant

An antioxidant can be broadly defined as any substance that delays or inhibit oxidative damage to a target molecule.The main characteristic of an antioxidant is its ability to trap free radicals.In plants, phenolics can act as antioxidant by donating electrons to guaiacol type peroxidases for the detoxification of H2O2 produced under stress conditions phenolics also provide protection against UV radiation through their potent radicals scavenging ability. Antioxidants are compounds in foods that scavenge and neutralise free radicals. Evidence suggests that antioxidant supplements do not work as well as the naturally occurring antioxidants in foods such as fruits and vegetables.

Sources of antioxidants:

- Allium sulphur compounds – leeks, onions and garlic.
- Anthocyanins – eggplant, grapes and berries.
- Beta-carotene – pumpkin, mangoes, apricots, carrots, spinach and parsley.

Definition:

Antioxidants are compound that inhibit oxidation, a chemical reaction that can produce free radicals and these start chain reaction that damage cells. Antioxidants terminate these chain reaction by removing free radical intermediates and inhibit other oxidation reaction.

Source of Antioxidants in plants:

They are most abundant in fruits and vegetables, as well as other foods including nuts, whole grains and meat, poultry and fish these are rich in phenolic compounds and carotenoids.

Role of antioxidant:

Antioxidant is molecules in our body which fight free radicals. Free radicals are molecules that can cause damage if the body has too high level of them. Antioxidants are molecules that neutralize free radicals, the molecules are unstable and can damage the cells.In addition to crucial roles in defence systems and as enzymes cofactors, antioxidants influence higher plant growth and development by modifying processes from mitosis and cell elongation to senescence and death.

Classification:

Antioxidants are classified in to two types they are Enzymatic Antioxidants and None – Enzymatic antioxidant. Enzymatic antioxidants consists of Superoxide dismutase & Gluthione peroxidase. None-Enzymatic antioxidants consists of Vitamin E, A, C & Flavonoids, Cartenoids etc.

Benefits and uses:

Brightness the skin.

Protects the skin from harmful UV rays.

They reduce oxidative stress.

They support disease prevention.

They aid in brain function,

They can contribute to mental health.

They can keep the skin healthy.

They reduce inflammation.

Brightness the skin

They aid in brain function

Reduce Inflamation

Three types of Antioxidants:

Antioxidants can be divided into three groups by their mechanism: (1) primary antioxidants, which function essentially as free radical terminators (scavengers); (2) secondary antioxidants, which are important preventive antioxidants that function by retarding chain initiation; and (3) tertiary antioxidants, which are concerned with the repair of damaged biomolecules.

Lawsonia Inermis :

Medicinal plants also called medicinal herbs, have been discovered and used in traditional medicine practice since prehistoric times. Plants play a major role on the earth and human beings depend on plant because of their medicinal properties. About 80% of the world population using plants as a medicinal drugs because plants have no side effect and shows synergistic effect unlike modern medicine.

Lawsonia inermis plant is mainly present in subtropical and tropical areas and is used in all over world. Traditionally, in Asian countries like India and Pakistan, plantleaves are applied to hands, hairs and feet. It has been used for over 9000 years for their cosmetic value as a dye.

This plant is mostly used for the treatment of High fever, Relieves Headache, Sore throat treatment Dye plant (red colour). Dye plant is used on the hands and on feets because this lawsonia inermis leaves helps in removing heat from the body so that's the reason it helps for the treatment of high fever and in Rainy season the human beings should not apply lawsonia inermis leaves paste on the hands because it causes cold infections.

Biological source: Lawsonia inermis L is commonly known as Henna.

Family: Belongs to the Lythraceae family and is the sole species in the genus.

Geographical source: A native of north Africa and south-west Asia, Australia in semi-arid zones and tropical areas the plant is now widely cultivates throughout the tropics as an ornamental and dye plant.

Synonym:

Telugu: Gorintaku

Hindi : Mehendi

English : Henna

Malyalam: Mignonette tree

Punjab: Nakrize, panwar

Sind: Mendi

Systemic positions:

Kingdom	Plantae
Family	Lythraceae
Order	Myrtals
Subfamily	Lythroideae
Genus	Lawsonia
Clade	Rosids,Angiosperms

Botanical description:

Leafs are small, opposite in arrangement along the branches, sub-sessile, about 1.5 to 5 cm long, to 2 cm wide, greenish brown to dull green, elliptic to broadly lanceolate with entire margin, petiole short and globorus and acute or obtuse apex with tapering base.It is a biennia dicotyledonous herbaceous shrub. A branched globrous shrub or small tree (2 to 6 m in height).

Stems are evergreen shrub or tree 2–7 m high; young stems 4-sided, sometimes forming rigid spines Lawsonia inermis is a much-branched glabrous shrub or small tree 2-6 m in height, which may be spiny. Bark greyish-brown, unarmed when young, older plants with spine-tipped branchlets. Young branches quadrangular, green but turn red with age.

Flowers have four sepals and a 2 mm (0.079) in calyx tube, with 3mm (0.12) in spread lobes. The ovary is four – celled, 5mm (0.20) in long and errect. Its petals are ovate with white or red stamens found in pairs on the rim of the calyx tube. Frits are small, brownish capsules, 4-8 mm (0.16 – 0.31 inches) in diameter, with 32-49 seeds per fruits, and open irregularly into four splits.

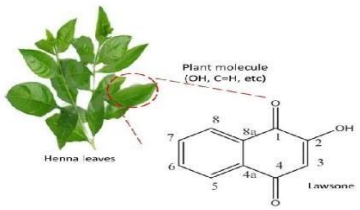
Pharmacological actions:

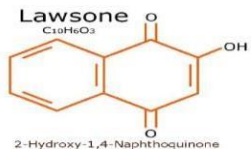
Antioxidant	Anticarcinogenic	Antimalarial
Antimicrobial	Antibacterial	Antifungal
Antiinflammatory	Antifertility	Antirypanosomal

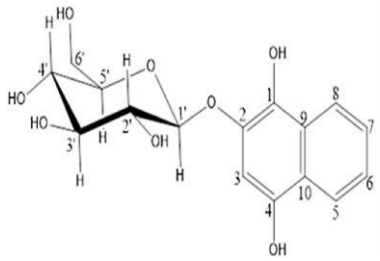
Uses:

- Ulcers in the stomach or intestines.
- Cancer
- Severe diarrhea caused by parasites called amoebs (amoebic dysentery).
- Enlarged spleen.
- Yellow skin (jaundice).
- Skin conditions, when taken by mouth.
- Headache.
- Dandruff, when applied to the scalp.

Chemical structures of Lawsonia inermis







Phenolic glycoside

MATERIALS & METHODS

Collection & Identification:

The proposed material for study was identified and submitted as Lawsonia inermis L stem and it as authenticated by Dr. K . Madhava chetty. Assistant professor, Department of Botony, Sri venkateshwara university, Tirupati and voucher no. 0397.Lawsonia inermis stem were collected from around Tirupati – 517 502 on 28/09/2023. The stem were collected washed with water, dried in sunlight and stored properly. The dried stem was powderded. Coarse powder was used for Maceration method, soxhilation extraction method, phytochemical analysis, HPLC,UV, In Vitro Antioxidant studies: 1.DPPH assay 2.Superoxide radical by Alkaline DMSO 3. Hydroxyl radical scavenging activity 4.Hydrogen peroxide scavenging activity.5.Ferric reducing antioxidant power assay.6 Statistical analysis.

Pharmacognostical study

Macroscopy:

The term macroscopy refers to things that can be seen with the naked eye.The macroscopical charecters like size, shape, base, surface, color, odor and taste of Lawsonia inermis.

Microscopy:

The term microscopy refers to object that are so small that they can be observed only with the help of microscope. The required samples of Lawsonia inermis L. stem were sectioned with the help of fresh blade. The sections were first cleared with chloral hydrate and then stained with phloroglucinol and concentrated HCL sections were also stained with Iodine solution (I-KI) for starch.Photographs were collected or taken with Binocular microscope observation.

Maceration studies:

Maceration is one of the simplest extraction technique in which coarse and powdered plant material is soaked such as methanol, ethanol, ethyl acetate, acetone, hexane etc.It is one of the popular and inexpensive technique used for the extraction of different bioactive compounds from plant material.

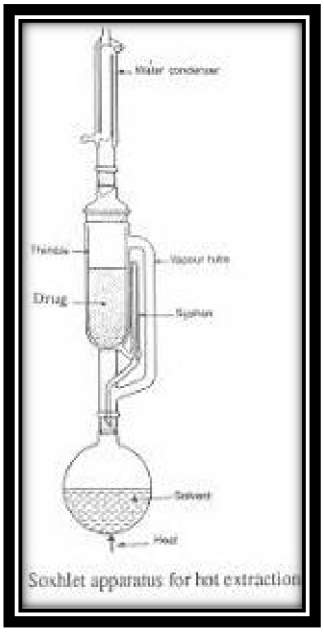
Solvents used:

- Water
- Ethanol
- Methanol
- Chloroform

Procedure :

Fresh stem of Lawsonia inermis were collected & dried for 3-5 days under the sunlight. Dried stem are grinded to convert into powder. weight the 10 gm of dried powder of (LI) stem and add 10 gm of powder into each 200 ml of water, ethanol, methanol, chloroform solvents mix each solvent with L I stem powder & cover them with a Aluminium foil paper.keep it a side for 48 hours and filtrate it.

Soxhletion method of ethanol extraction



Plant material can be fresh (for example, a plant leaf) or dried. It needs to be crushed, using a pestle and mortar, to provide a greater surface area. The plant material should be sufficient to fill the porous cellulose thimble (in our experiments we use an average of 14 g of thyme in a 25- x 80-mm thimble).

Following this, the solvent (250 ml of ethanol) is added to a round bottom flask, which is attached to a Soxhlet extractor and condenser as shown in the figure on an isomantle. The crushed plant material is loaded into the thimble, which is placed inside the Soxhlet extractor. The side arm is lagged with glass wool. The solvent is heated using the Isomantle and will begin to evaporate, moving through the apparatus to the condenser. The condensate then drips into the reservoir containing the thimble. Once the level of solvent reaches the siphon it pours back into the flask and the cycle begins again. The process should run for a total of 16 hours. The equipment can be turned on and off when overnight running is not permitted, and the time split over a number of days.

Preliminary phytochemical screening

The EELI (Ethanolic extract of lawsonia inermis) obtained was subjected to different qualitative chemical tests for the identification of different chemical constituents.

Detection of Alkaloids:

Dragendroff's reagent:

A few mgs of EELI in active acid or HCL was treated with two drops of Dragendroff's reagent (potassium mercuric iodide).

Wagner's reagent:

A few mgs of EELI was treated with two drops of wagners reagent.

Hagers reagent:

A few mgs of EELI was treated with two drops of Hagers reagent.

Acids:

A few mgs of EELI was treated with aqueous NaHCO3.

Detection of Coumarin :

A few mgs of the EELI in alcohol was treated with alcoholic NaOH.

Detection of Carbohydrates:

A few mgs of the EELI was dissolved in suitable solvent and filtered. The filtrate was subjected to the following tests.

Molish Test:

The filtrate was treated with 2-3 drop of 1% alcoholic naphthol and 3 ml of conc. H2SO4 was added along with the sides of the test tube.

Fehling Test:

The filtrate was first treated with 1 ml of fehlings test solution and heated.The reddish or orange precipitate was obtained. The red precipitate indicates the absence of reducing sugars.Another potion of liquid hydrolysate was solicated to "Legals" and Borntragers" test.

Legals Test:

To the hydrolysate 1 ml of sodium nitro prusside solution was added and it was made alkaline with NaOH solution.

Brontragers Test:

The hydrolysate was treated with chloroform layer was separated. Equal quantity of dil.Ammonia solution was added to it.

Detection of Fixed oils & fats:

A few mgs of EELI was pressed separately between two filter papers. If charecteristic oil stain was observed.

Phenolphthalein Test:

A few deops of alcoholic KOH added to the EELI with a few drops of phenolphthalein. The mixtures was heated on a water bath for one to two hours. The formation of soap was observed.

Flavonoids:

Shinoda Test:

A few mgs of EELI was dissolved in alcohol separately and was treated with magnesium foils and a few drops pf consentrated Hydrochloric acid.

- A few mgs of EELI was dissolved separately in alcohol and to this a Magnesium metal, followed by conc. Hydrochloric acid added to the solution
- A small portion of the EELI was dissolved separately in alcohol. The EELI was yellow in colour which on addition of acid becomes colourless.

Detection of Furonoids :
Ehelich Test:

A few mgs of EELI was dissolved separately in alcohol and was treated with a pinch of para dimethyl amino-benze aldehyde and a few drops of hydrochloric acid.

Detection of Phenols:

A few mgs of EELI was dissolved separately in alcohol and was treated with alcoholic ferric chloride.

Detection of Phytosterols:

A few mgs of EELI was dissolved in 5 ml of chloroform separately. Then this chloroform solution was subjected to a libermans- buchard test. The above prepared chloroform solution was treated with a few drops of conc. Sulphuric acid followed by 1 ml of active anhydride solution. This solution was heated gently if necessary.

Salkowskis Test:

To 1 ml of above prepared Chloroform solution and a few drops of conc. H_2SO_4 was added.

Detection of Saponins :

A few mgs of EELI was diluted with 20 ml of distilled water and it was heated in a graduated cylinder for 15 minutes.

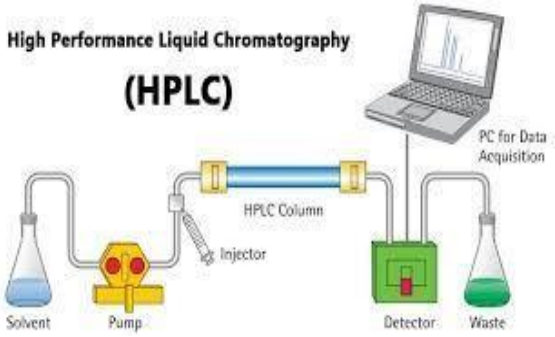
Detection of Tannins:

A few mgs of EELI was dissolved in alcohol separately and it was treated with a few drops of aqueous lead solution.

Detection of Triterpenoids:

A few mgs of EELI was taken in a dry test tube and it was treated with a bit of tin foil and 0.5 ml of thionyl chloride. Heated gently if necessary.

High Performance Liquid Chromatograhy



Standard Solution Preparation:

Accurately weigh and transfer 10mg of Sample into a 10ml clean dry volumetric flask add about 30ml of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. Sonicate the Solution upto 30 min after Sonication filter the Solution with 0.44 micron nylon injection filter and injected in to HPLC System.

Chromatographic Conditions:

Instrument used	:	Waters HPLC with auto sampler and PDA or detector.
Temperature	:	Ambient
Column	:	Inertsil ODS (4.6 x 150mm, 5µm)
Buffer	:	0.1% Acetic acid
PH	:	3.0
Mobile phase	:	30% buffer 70% Methanol
Flow rate	:	1 ml per min
Wavelength	:	270-290 nm
Injection volume	:	20 µl
Run time	:	10min.

UV Spectroscopy

Instrument Performance

Model: UV-VIS Spectrophotometer

Number: 18-1885-01-0115

Spectral Band width: 2.00 nm

Scan Spectrum Performance

Scan Range: 200.00 to 400.00 nm Measure Mode: Abs

Interval: 5.00 nm

Speed: Fast

Data File: Ethanol Extract.spd

Create Date/Time: Monday, April 22, 2004 1:45:40 PM

Data Type: Original

Method File:

Analyser: Administrator

Sample name: Ethanol extract

In vitro – Antioxidant activity

The medicinal properties of plants have been investigated in the recent scientific development throughout the world, due to their potent antioxidant activities, no side effect and economic viability. Poly phenol compounds such as flavonoids and phenolic groups widely distributed in plants which have been reported to exert multiple biological effect, including antioxidant, free radical scavenging abilities, anti-inflammatorya and anti tumor.

Evaluation of free radical scavenging potential by In vitro models:

1. DPPH radical scavenging assay
2. Superoxide radical by Alkaline BMSO
3. Hydroxyl radical scavenging activity
4. Hydrogen peroxide scavenging activity
5. Ferric Reducing Antioxidant power assay
6. Statistical analysis

DPPH radical scavenging assay

The effect of EELI on DPPH radical was determining using the method of Liyana Pathiranan & Shahidi. A solution of 0.135 mm DPPH in methanol was prepared&1.0 ml of this solution was mixed with 1.0 ml of EELI in methanol containing 0.02 – 0.1 mg of the EELI. The reaction mixture was vortexed thoroughly and left in thedark at roo, temperature for 30 min. The absorbance of the mixture was measured spectrophotometrically at 517 nm. Quercetin was used as standard.The ability toscavenge DPPH radical was calculated by the following equation DPPH radical scavenging activity

$$(\%) = [(\text{Abs control} \;\; \text{Abs sample})]/ (\text{Abs control})] \times 100$$

where, Abs control DPPH radical is the absorbance of DPPH radical + methanol;Abs sample is the absorbance of + sample extract/standard.

Superoxide radical by Alkaline DMSO

Preparation of Test and Standard solutions:

10mg of EELI and the standard (Quercetin) were weighed accurately and separately dissolved in 1mL of DMSO. These solutions were serially diluted with DMSO to obtain the lower dilutions.

Procedure:

To the reaction mixture containing 1 ml of alkaline DMSO, 0.3 ml of the EEL and standard were added in DMSO at various concentrations followed by 0.1 ml of NBT (0.1 mg) to give a final volume of 1.4 ml. The absorbance was measured at 560 nm.

$$(\%)\ \text{inhibition} = [(\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}})]/(\text{Abs control})] \times 100$$

Hydroxyl radical scavenging activity

The reaction mixture contained 0.1ml of deoxyribose, 0.1ml of $FeCl_3$, 0.1ml of EDTA, 0.1ml of H_2O_2, 0.1ml of ascorbate, 0.1ml of KH_2PO_4-KOH buffer and 20µl of EELI in a volume of 1.0 ml. The mixture was incubated at 37^{o}C for 1 hour. At the end of the incubation period, 1.0 mlof TBA was added and heated at 95℃ for 20 minutes to develop the color .After cooling the thiobarbituric acid reactive substances (TBARS) formation was measured spectrophotometrically V at 532 nm against blank. The hydroxyl radical scavenging activity was determined by comparing the absorbance of the control with that EELI.

$$(\%)\ \text{inhibition} = [(\text{Abs control} - \text{Abs sample})] / (\text{Abs control})] \times 100$$

Hydrogen peroxide scavenging activity

1.0 ml of hydrogen peroxide (20 mm) was prepared in phosphate duffered saline (PBS, PH 7.4). 1.0 ml of various concentration of the EELI or standards in methanol was added to 2ml of hydrogen peroxide solution in PBS.Then finally the absorbance was measured at 230nm after 10 minutes. The percentage inhibition was calculated using equation.

$$(\%)\ \text{inhibition} = [(\text{Abs control} - \text{Abs sample})]/ (\text{Abs control})] \times 100$$

Ferric Reducing Antioxidant Power Assay (FRAPA)

Different concentration of the EELI was performed (20-100µg/ml) in 0.2 m phosphate buffer PH, 6.6 containing 1% ferrocyanate. The mixture was incubated at 50℃ for 20 minutes.10% trichloroacetic acid (TCA, 2.5 ml) was added to a portion of this mixture (5ml) and centrifuged at 3,000 g for 10 minutes. The supernatant was separated and mixed with distilled water (2.5 ml) containing 1% ferric chloride (0.5 ml). The absorbance of this mixture was measured at 700 nm. The intensity in obsorbance could be the measurement of antioxidant activity of the extract.

$$(\%)\ \text{inhibition} = [(\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}})]/(\text{Abs control})] \times 100$$

Statistical analysis: The results were expressed IC50 and correlation coefficient (R) value of FRAP. It was calculated by using graph pad prism version 5 and correlation analysis of FRAP was carried out using the correlation and regression program.

In vivo Anti-inflammatory Activity:

The in vivo anti-inflammatory activity of extract based was determined using the carrageenan-induced paw edema method and compared with the marketed formulation according to previous reports.

The animals were divided into three groups of 6 animals. Briefly, the first group (control) received carrageenan only without the drug.

The edema was induced to the second (test) and third (standard) group animals in the right hind paw using 0.1mL (1% w/v) carrageenan injection in the sub-plantar region.

Then Cream and standard marketed gel were applied topically to the right hind paw of test and control group animals respectively 60 min before the administration of carrageenan injection.

The paw edema volume was measured after (0, 0.5, 1, 2, 3, and 4h) of carrageenan injection using a digital plethysmometer. Finally, the percentage inhibition of paw volume for treated groups was determined by comparing with the control group mean paw volume.

RESULTS

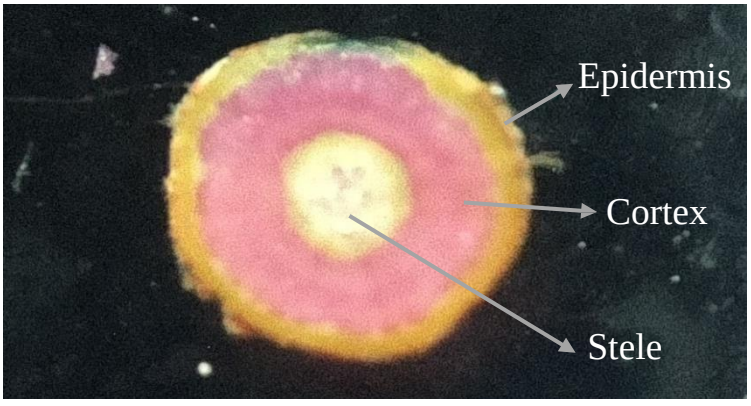
Pharmacognostical study

Macroscopical characters:

The stem of Lawsonia inermis L is a much – branched glabrous shrub or small tree 2-6 m in height, which may be spiny. It is ruff like structure .Bark greyish-brown, unarmed when young, older plants with spine-tipped brachlets. Young branches quadrangular, green but turn red with age.This is the photograph of lawsonia inermis stem.

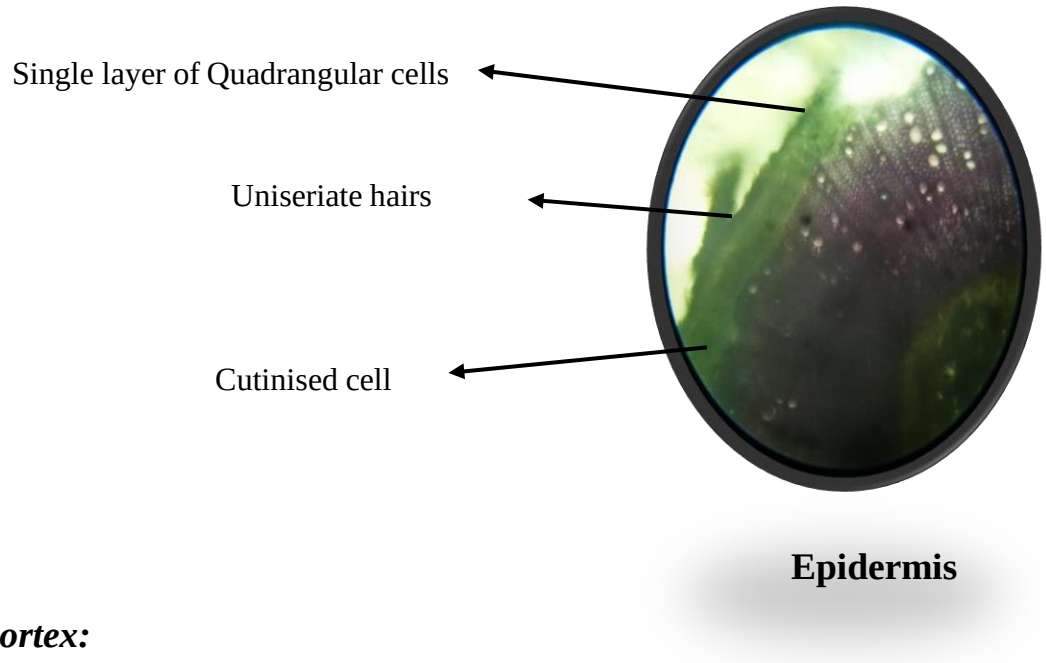
Microscopical charecters:

A transverse section of stem from lawsonia inermis plant is circular in outline and differentiated into three regions they are epidermis, cortex, and stele.



Epidermis:

It is the outermost layer and consist of single layer epidermis of quadrangular cells. The outer walls of the cells are cutinised. From some of the cells multicellular, uniseriate hairs called epidermal hairs, (Trichomes) are formed. Stomata are present.



Epidermis

Cortex:

It is the middle region and consists of three parts they are Hypodermis, Inner cortex and Endodermis

Hypodermis;

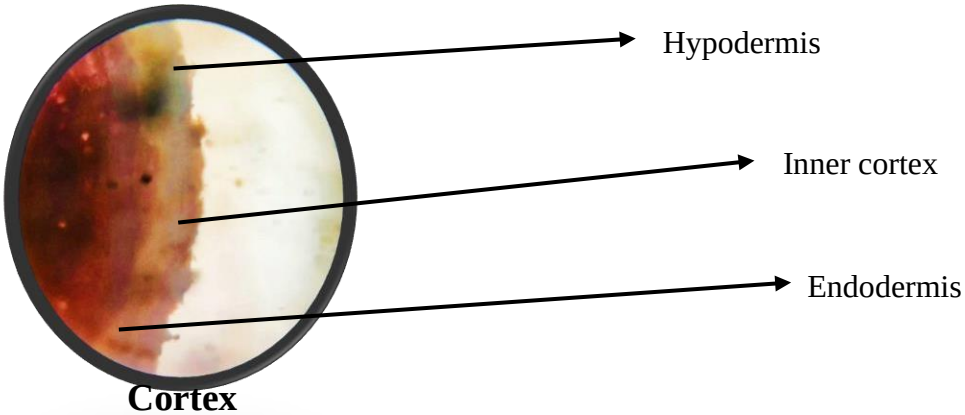
Below the epidermis 6-7 layerd collenchymatous hypodermis is present. It gives mechanical support.

Inner cortex:

It consists of 7-10 layer of loosely arranged parenchymatous cell filled with chloroplasts. In this region small mucilage ducts are present.

Endodermis:

Below the inner cortex single layered endodermis is present Endodermis cells contain starch grains. So called starch sheath.



Cortex

Stele:

Stele is the inner region and occupies relatively larger region in stem. It consists of pericycle, vascular bundles pith & medullary rays.

Pericycle is present as 3 to 6 layered sclerenchymatous patch above each vascular bundle with intervening masses of parenchyma.

Vascular bundles are 15-20 in number and are arranged in a single ring (Eustele).

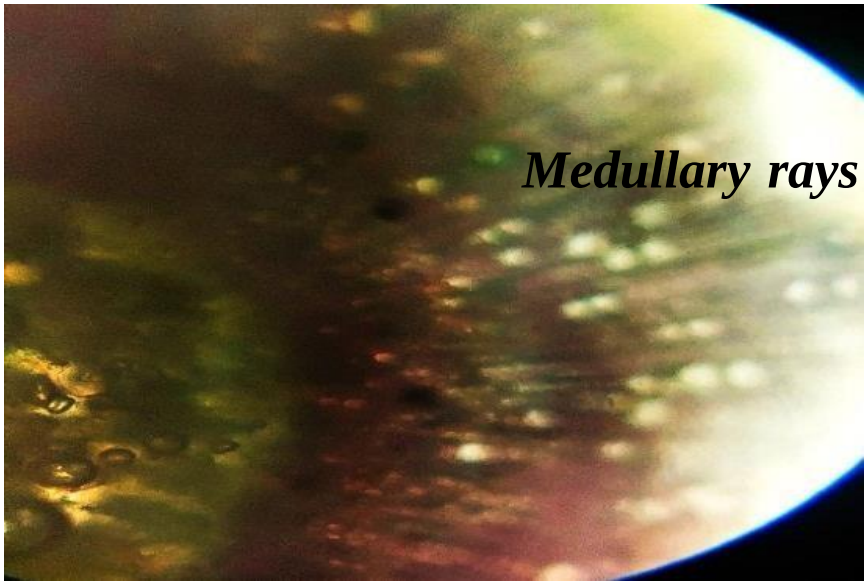
They are top shaped, conjoint, collateral and open.

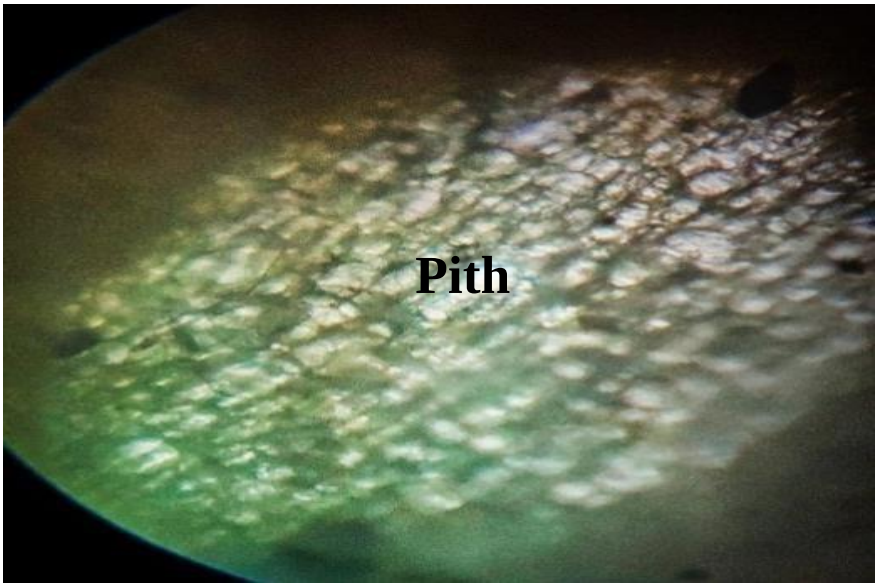
Pith:

Central part of the stem is occupied by parenchymatous pith. After pith region xylem and phloeam are present.

Medullary rays:

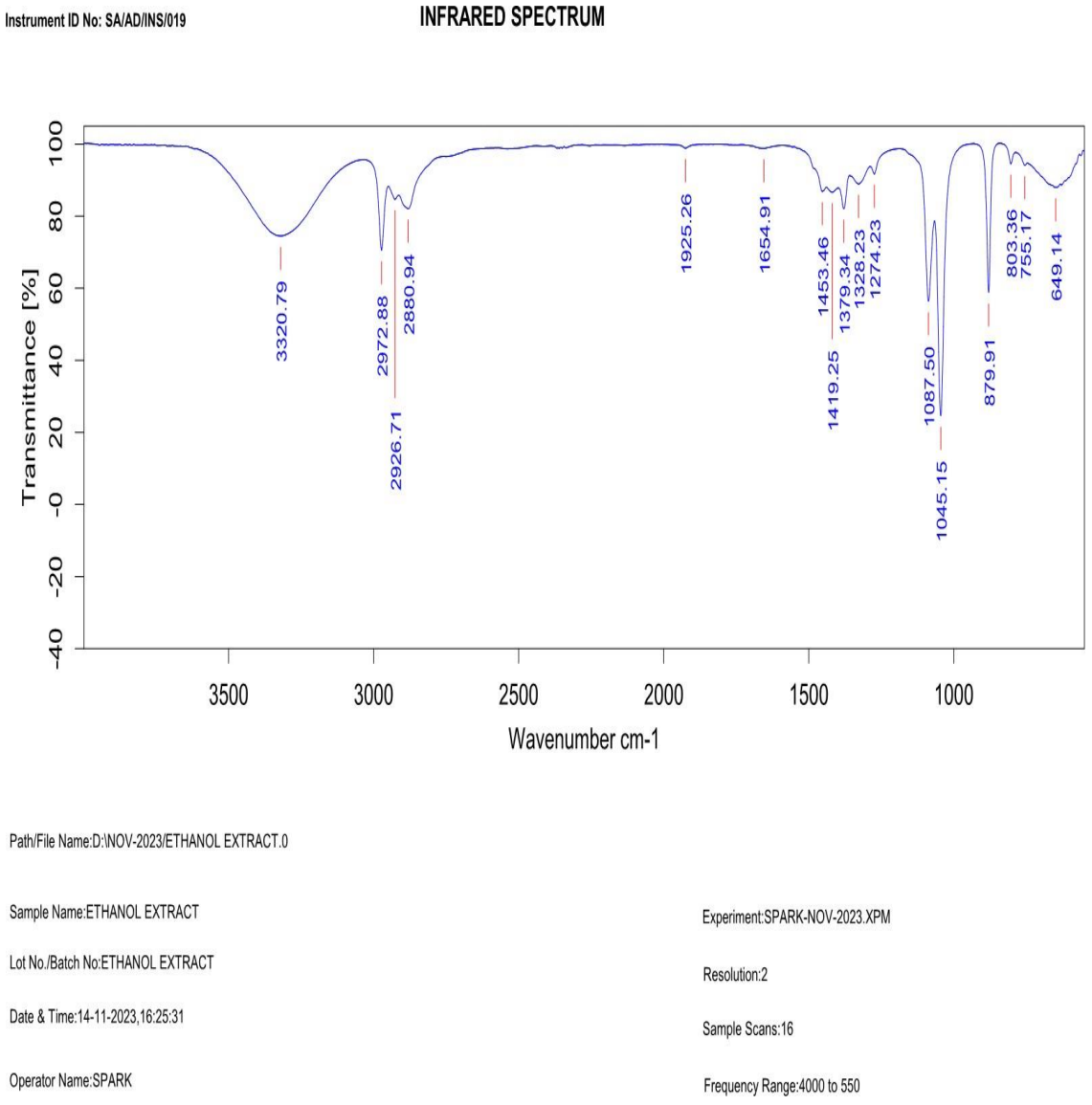
Parenchymatous tissue present between vascular bundles helps in lateral conduction medullary rays araising from pith region





Maceration:

After filtering all solvents ethanol solvent got more solvent extract compare to other solvent extracts.



Graph 1

Soxhlation extraction method

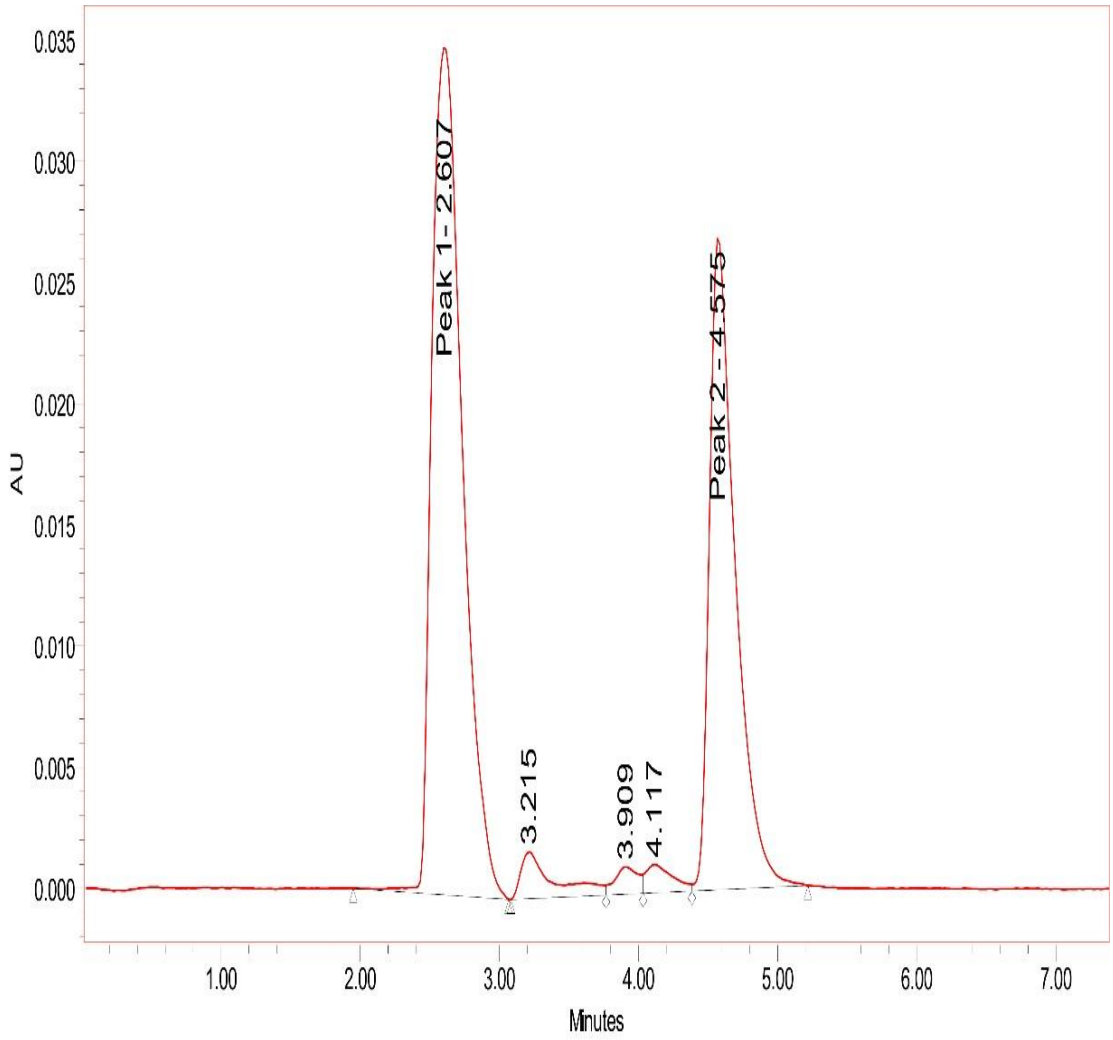
Once the process has finished, the ethanol should be evaporated using a rotary evaporator, leaving a small yield of extracted plant material (about 2 to 3 ml) in the glass bottom flask.
This yield can be used for Antimicrobial activity.

Phytochemical analysis

Phytochemicals	Ethanol extract
1) Detection of alkaloids	
• Dragendroff's reagent	+
• Wagners reagent	+
• Hagners reagent	+
2) Acid test	+
	+
3) Detection of coumarin	
	+
4) Detection of Carbohydrates	
	+
i) .Molisch test	-
ii) Fehling test	-
iii) legals test	-
iv) Brontragers test	
5) Detection of fixed oils & fats	+
* Phenolphthalein test	+
6) Flavonoids	
* Shinoda test	+
7) Detection of furonoids	
* Ehelich test	+
8) Detection of phenols	+
9) Detection of phytosterols	+
* Salkowskis test	+
10) Detection of saponins	+
11) Detection of Tanins	+
12) Detection of Triterpenoids	+

Table 1

High Performance liquid chromatography

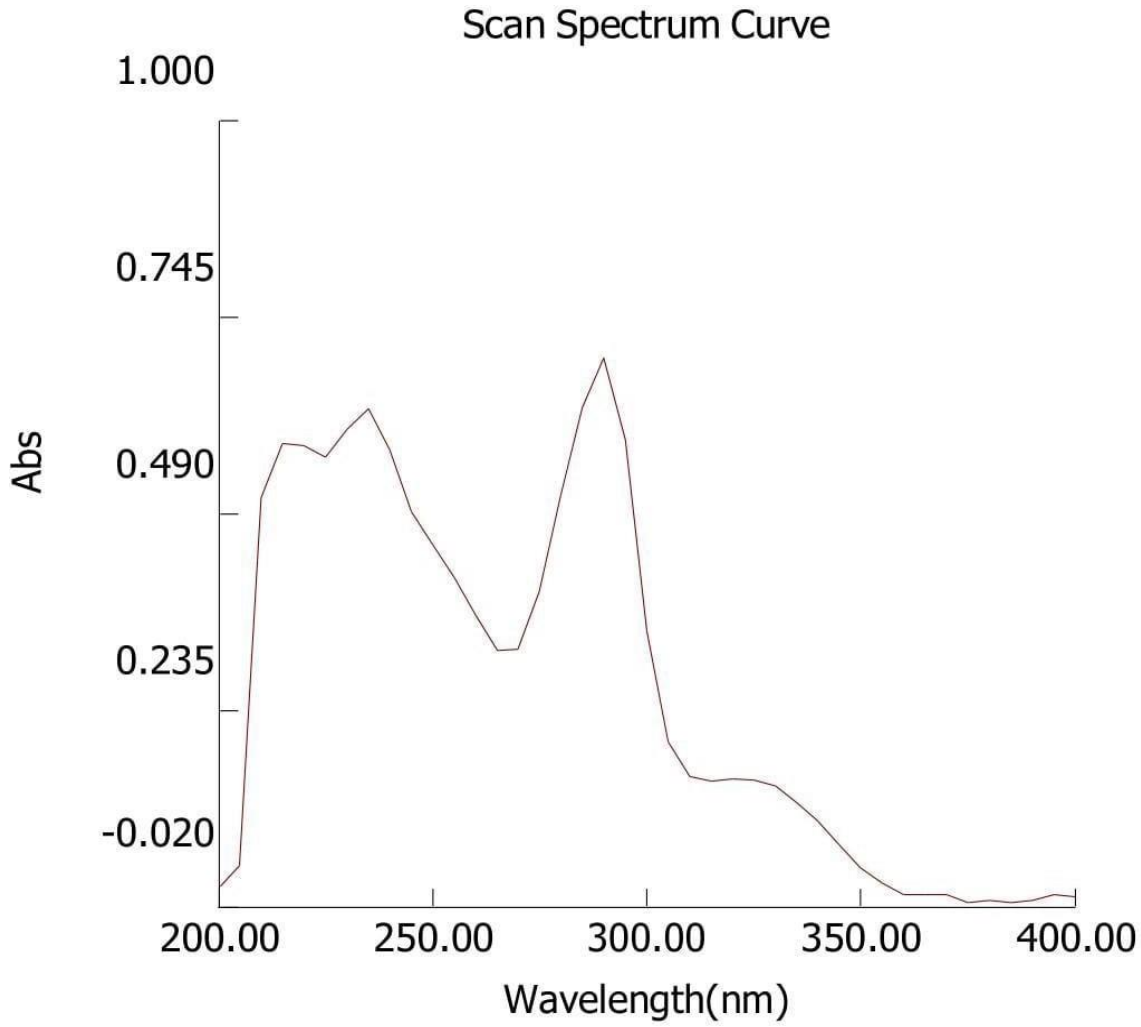


	Peak Name	RT	Area	Height	USP Plate Count	USP Tailing	USP Resolution	% Area	Purity1 Angle	Purity1 Threshold
1	Peak 1	2.607	537299	35005	712.1	1.5		56.92		
2		3.215	31400	1932	1978.4		1.8	3.33		
3		3.909	12996	1128	1534.8		2.0	1.38		
4		4.117	15466	1166	1203.8		0.5	1.64		
5	Peak 2	4.575	346853	27023	3022.8	1.7	1.1	36.74		

Graph 2

UV Spectroscopy

Ethanol Extract. spd Ethanol Extract.spd Spectrum

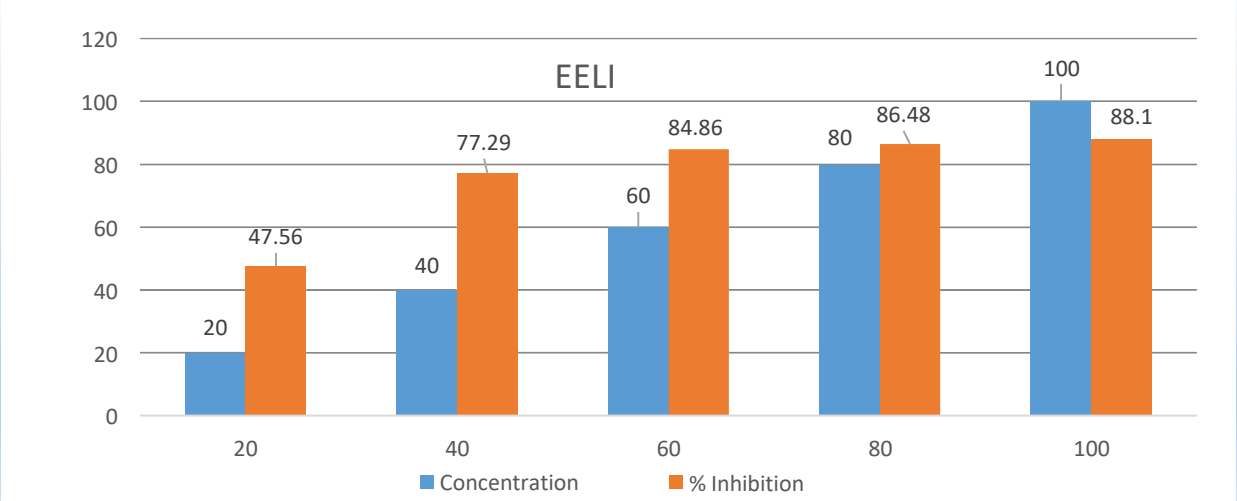


No.	P/V	Wavelength(nm)	Abs	Comment
1	Peak	390.00	-0.005	
2	Peak	280.00	0.373	
3	Peak	265.00	0.179	
4	Peak	220.00	0.107	

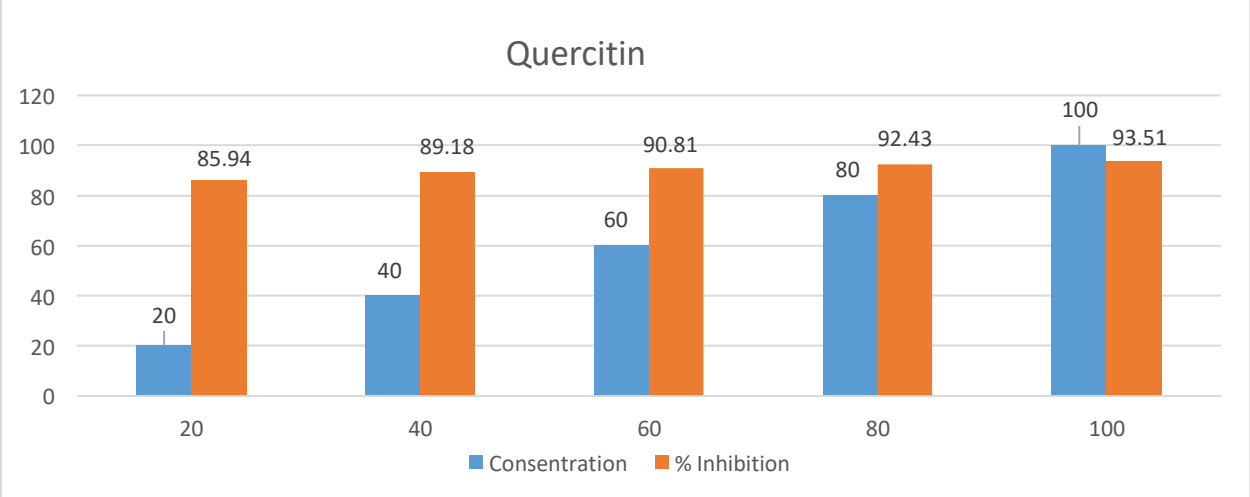
Evaluation of free radical scavenging potential by In vitro models:

Effect of EELI on DPPH method:

Treatment	Conc(µg/ml)	%Inhibition
EELI	20	47.56
	40	77.29
	60	84.86
	80	86.48
	100	88.10
Quercetin	20	85.94
	40	89.18
	60	90.81
	80	92.43
	100	93.51
IC50Quarcetin0.455µg/ml IC50 EELI 20.1µg/ml		



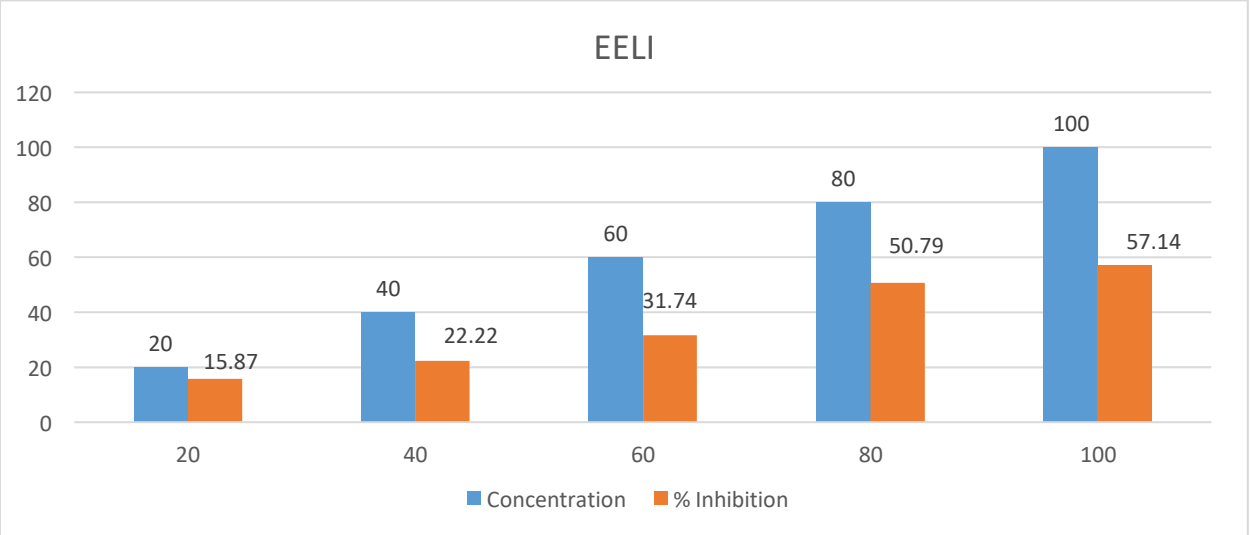
Graph 3



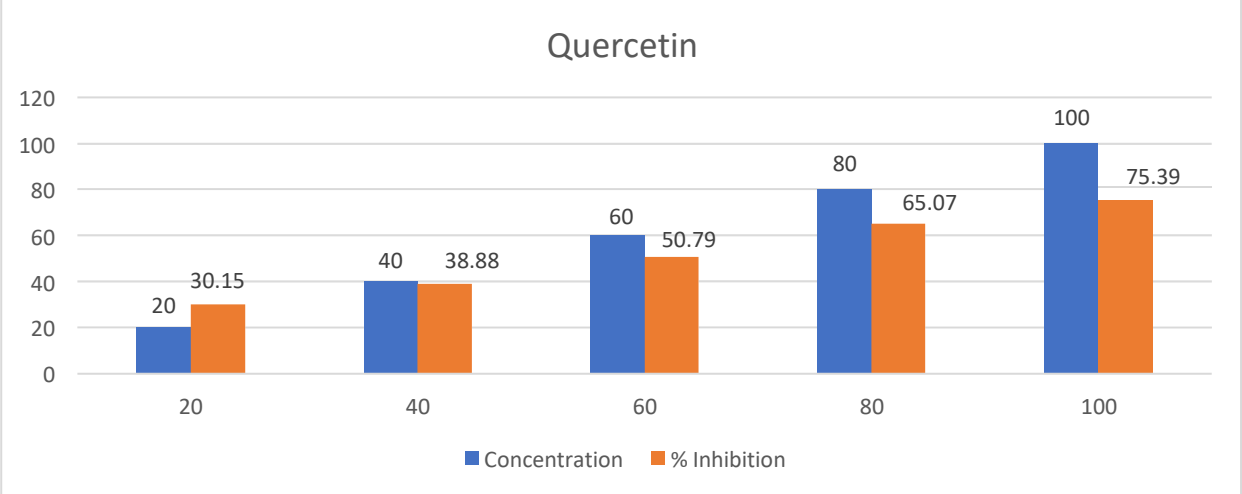
Graph 4

Effect of EELI on Superoxide radical scavenging activity:

Treatment	Conc(µg/ml)	%Inhibition
EELI	20	15.87
	40	22.22
	60	31.74
	80	50.79
	100	57.14
Quercetin	20	30.15
	40	38.88
	60	50.79
	80	65.07
	100	75.39
IC50Quarcetin49.64µg/ml IC50 EELI 20.1µg/ml		



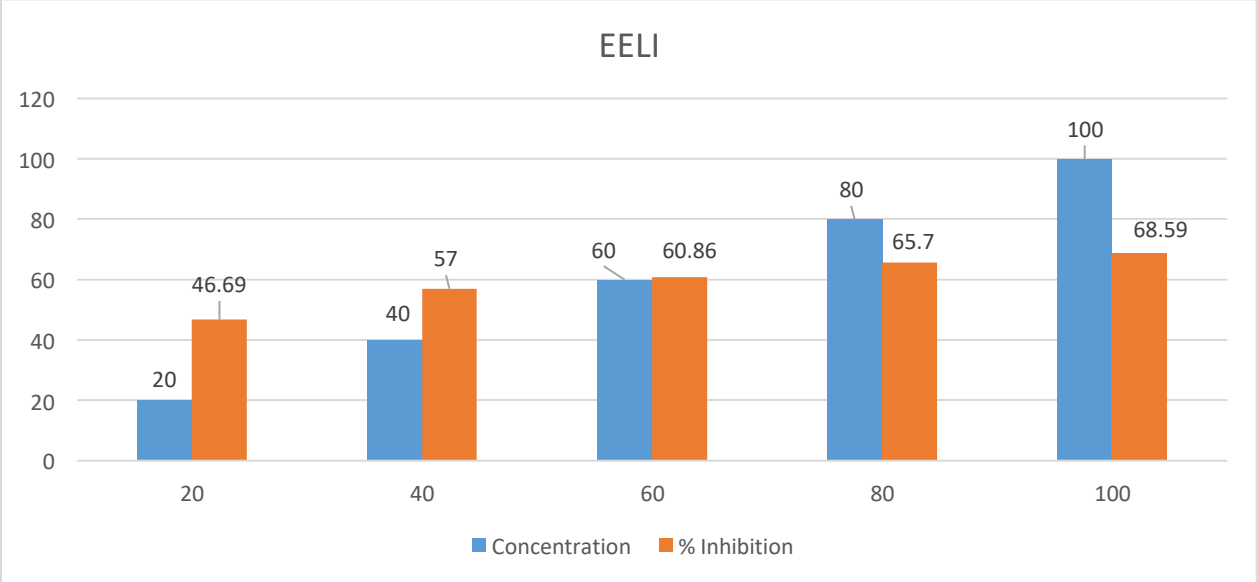
Graph 5



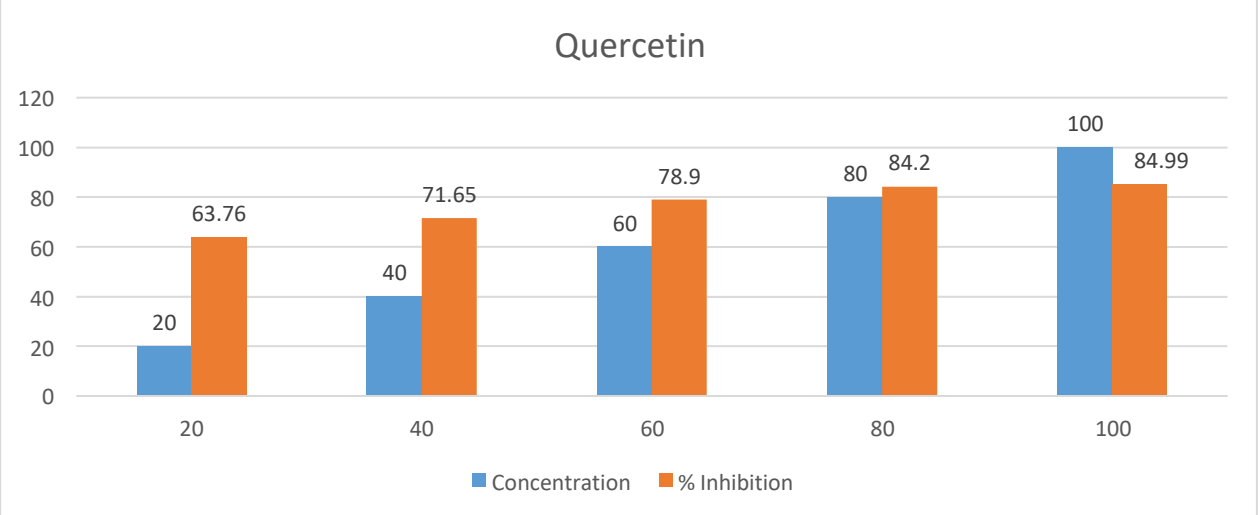
Graph 6

Effect of EELI on Hydroxyl radical scavenging activity:

Treatment	Conc(µg/ml)	%Inhibition
EELI	20	46.69
	40	57.00
	60	60.86
	80	65.70
	100	68.59
Quercetin	20	63.76
	40	71.65
	60	78.90
	80	84.20
	100	84.99
IC50Quarcetin9.74µg/ml IC50 EELI 24.41µg/ml		



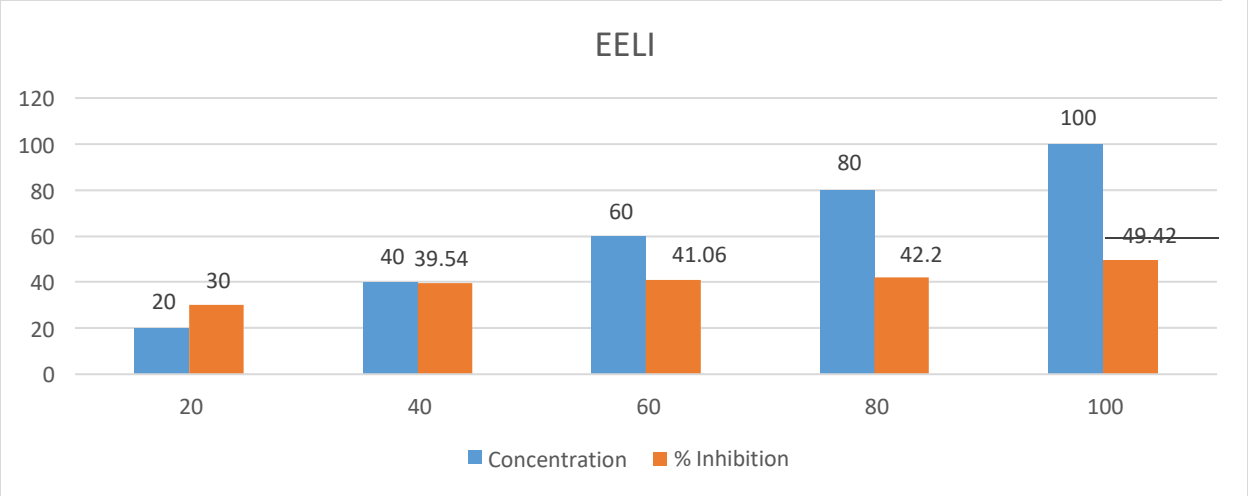
Graph 7



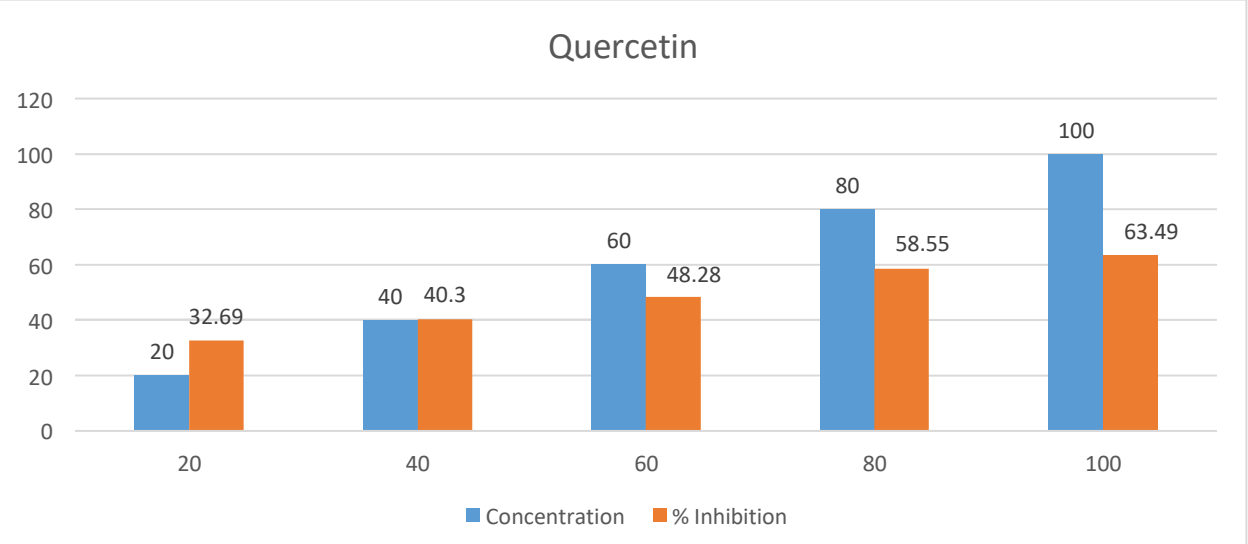
Graph 8

Effect of EELI on Hydrogen peroxide radical scavenging activity:

Treatment	Conc(µg/ml)	%Inhibition
EELI	20	30.00
	40	39.54
	60	41.06
	80	42.20
	100	49.42
Quercetin	20	32.69
	40	40.30
	60	48.28
	80	58.55
	100	63.49
IC50Quarcetin54.67µg/ml IC50 EELI 121.8µg/ml		



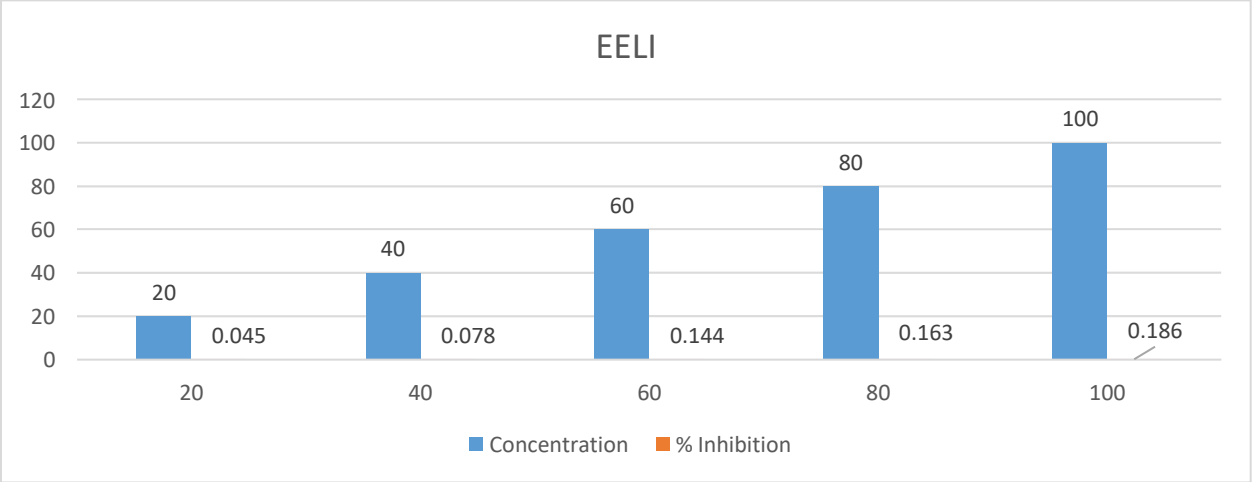
Graph 9



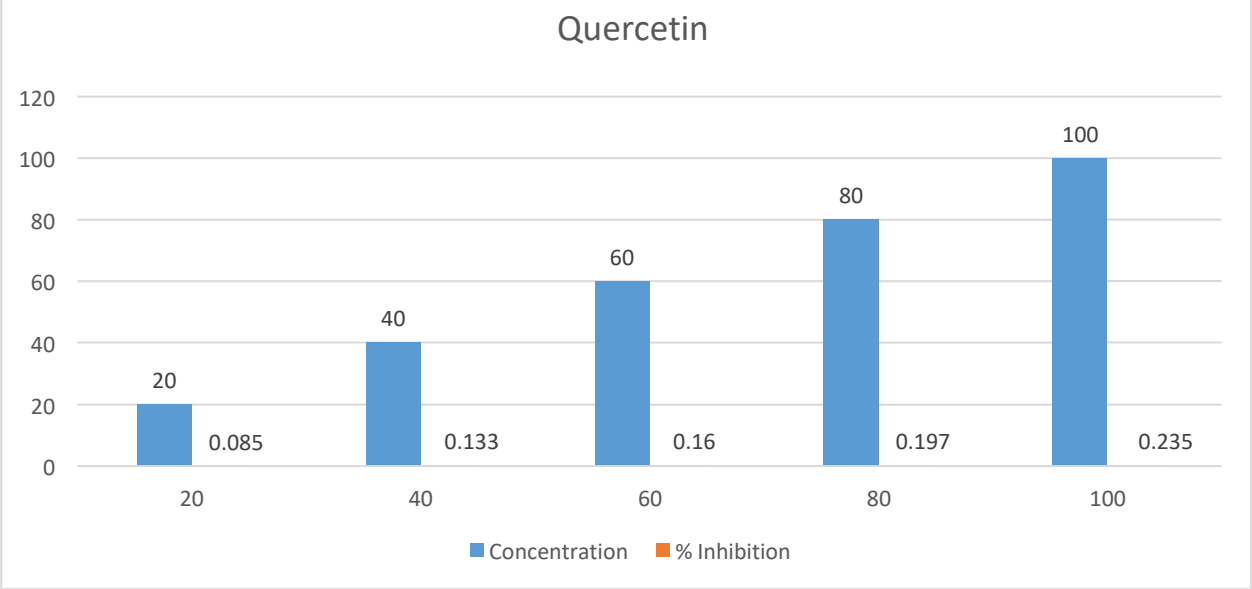
Graph 10

Effect of EELI on Ferric reducing antioxidant power assay:

Treatment	Conc(µg/ml)	Absorbance
EELI	20	0.045
	40	0.078
	60	0.144
	80	0.163
	100	0.186
Quercetin	20	0.085
	40	0.133
	60	0.160
	80	0.197
	100	0.235
Quarcetin r^2 0.9862 EELI r^2 0.9592		



Graph 11



Graph 12

In vivo Anti- inflammatory activity

The anti-inflammatory activity of LI stem extract was studied by using the prevention of carrageenan-induced paw edema model and was compared with marketed gel (standard). The injection of carrageenan developed marked edema. The application of LI stem extract resulted in significantly ($p<0.01$) more % inhibition of swelling of rat paw edema (37.68±0.73%) when compared to marketed gel (28.98±0.68%) at the end of 4h.

Thus, the substantially improved anti-inflammatory activity of LI stem extract could be attributed to the better permeation

CONCLUSION

In these present investigations various pharmacognostical standardization parameters such as macroscopy, microscopy, maceration, soxhlation extraction method & preliminary phytochemical screening, High perfomenced liquid chromatography, UV Spectroscopy In vitro Antioxidant studies were carried out which could be helpful in authentication of lawsonia inermis L stem. & In vivo Anti-Inflammatory activity of LI stem could be attributed to the better permeation. The result of the present study will also serve as reference material in the preparation of herbal monograph.

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